Virtual Cell Version 4.0

Facilitated Calcium Diffusion in the Intestinal Epithelial Cell

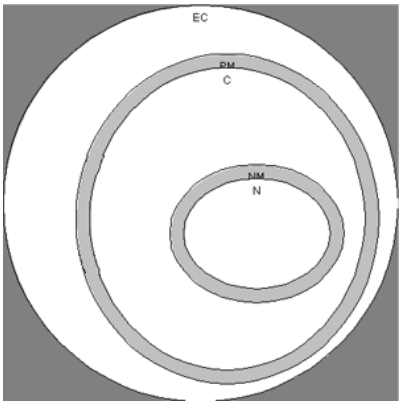
The Virtual Cell tutorial is designed to work in conjunction with the Virtual Cell User Guide. Although this model is somewhat complex for a first time user of the Virtual Cell software, it proves the capabilities of the software. This tutorial has been designed with the intention that you may use it to reproduce this model as your own or load in the public version. If you choose to load the public version, you must first go to View>Private Only and make sure this option is not selected. By default it will be selected. Select the Model workspace tab and in the Model Database panel go to the tutorial folder and load facilitated_ca_diffusion.

Physiological Basis

This model has been developed to study the role of calbindin in facilitated calcium diffusion in intestinal calcium absorption. Simulations also aid in the clarification of calcium steady-state levels within the enterocyte. The simplified model portrays calcium channels at the brush border membrane and calcium pumps at the basolateral membrane. Tight junctions depicted at the apical region of the cell are assumed to be completely impermeable to calcium diffusion. In addition, the model does not account for the paracellular transport of calcium into neighboring cells.
Refer to the Geometry document to preview the experimental image on which the model is based. A public Geometry document is available, epithelial_single, for use in this tutorial. The tutorial begins with defining the biological model and concludes with a spatial simulation.

BioModel

Cellular Structures



The software initiates with an undefined BioModel. Select the unnamed compartment once with the left mouse button. The compartment will turn red indicating the selection. Hold down the right mouse button and select Properties. Enter 'EC', for extracellular, in the text field and press OK.

Select the Feature Tool,  , once and then click in the extracellular compartment. A new compartment will appear and an Add New Feature dialog will automatically open. Type in 'C', for cytosol, in the Feature Name text field and type in 'PM' in the Membrane Name field for plasma membrane; press Add Feature.

Select the Feature Tool once again and click in the cytosolic compartment. Type in 'N', for nucleus, in the Feature Name text field and type in 'NM', for nuclear membrane, in the Membrane Name text field; press Add Feature to implement the names.

Molecular Species

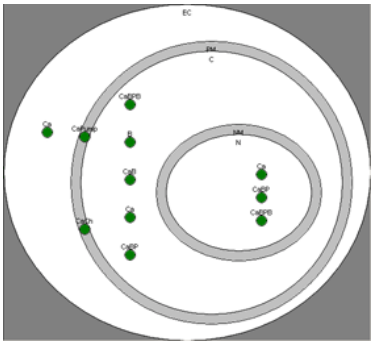
ExtraCellular

Select the Species Tool  and click once with the left mouse button in the EC compartment to open the Add New Species dialog. Type Ca, Calcium, in the text field; press Add to create the Species.

PlasmaMembrane

The apical calcium channel is represented as a species on the PlasmaMembrane. Click once on the Species Tool and click once on the PlasmaMembrane. Create the channel species as described above using CaCh for the name. Use the Species tool again to create the basal calcium pump on the PlasmaMembrane, name the pump CaPump.

Cytosol



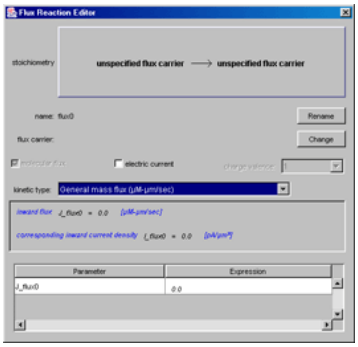
Add the following species to the cytosol compartment; calcium, calbindin, calbindin bound, endogenous buffer, and endogenous buffer bound. Calcium was already created, so you can use the Copy and Paste command via the right mouse button. Select the Species and go to Edit>Copy and then click in the Cytosol compartment and go to Edit>Paste. Continue with creating the new species as described before. Use the following names for the new species; Calbindin, CaBP, Calbindin bound, CaBPB, Endogenous Buffer, B, Endogenous Buffer Bound, CaB.

Nucleus

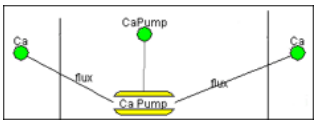
Continue to use the cut and paste feature of the Species Tool to add calcium, calbindin and calbindin bound to the nuclear compartment. At this point you should have generated a model that looks similar to the picture to the right.

Save the model, if you have not done so already, by going to File>Save As.

Biochemical Reactions
PlasmaMembrane



Select the PlasmaMembrane with the left mouse button. Use the right mouse button to access the Reactions submenu. Arrange the species icons in the Cytosol "C" Column such that each is visible. Select the Flux Tool  and then click once in the PlasmaMembrane "PM" column. The Flux Reaction Editor will automatically open. Press Rename to access the FluxReaction Name dialog. Enter Ca_channel in the text field; press OK to accept the name and to close the window. Click on Change and select calcium as the Flux Carrier and then click OK.



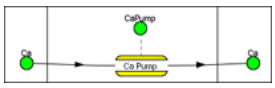
The software will automatically connect Ca_EC and Ca_C to the Flux Icon. Close the Flux Reaction Editor and use the line tool to connect CaCh to the middle of the Flux Icon. You will notice the word Catalyst will appear as you make the proper connection. It is important to make this connection prior to entering the rate equation otherwise CaCh_PM will be recognized as a parameter and not as a membrane species.

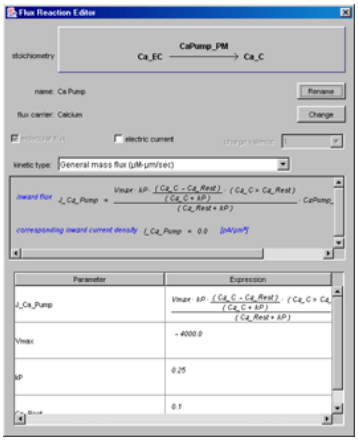
Reopen the Flux Reaction Editor by selecting the Flux icon and then use the right mouse button to access the Properties option. Select General mass flux for the Kinetic type. Define J_Ca_channel with the following equation:

(J0*Kc*(Ca_EC-Ca_C)/(Kc+Ca_C)*CaCh_PM)

Next define the parameters that have been introduced in the rate equation, J0, the flux density of an open channel and Kc, the dissociation constant for calcium binding to a channel. Double click in the Expression column for J0 and enter .014. This number was derived by dividing the FluxChannelCoefficient (.14) by the ChannelDensity (10). Double click the Expression column for Kc and enter 0.5 for its expression value. Close the Reaction Editor once you have entered all the rate information.

Select the Flux Tool once again and then click in the PlasmaMembrane column. Press Rename and name the flux reaction CaPump. Press Change to select calcium as the Flux Carrier. Once again notice the software will automatically connect Ca_EC and Ca_C to the Flux Icon. Close the Flux Reaction Editor and use the line tool to connect CaPump to the middle of the Flux Icon. You will notice the word Catalyst will appear as you make the proper connection. Remember it is important to make this connection prior to entering the rate equation. If you do not do this, CaPump_PM will not be recognized as a membrane species but as a parameter.





Select General mass flux for the kinetic type. Define J_Ca_Pump with the following equation:

((Vmax*kP*((Ca_CCa_Rest)/(Ca_C+kP))/(Ca_Rest+kP)*(Ca_C>Ca_Rest))*CaPump_PM)

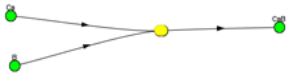
Enter -4000 for Vmax, the maximum pump turn over rate. Vmax must be entered as a negative value to allow for the flux from the cytosol into the extracellular region. Enter 0.25 for kP, the dissociation constant for calcium binding to the pump and 0.1 for Ca_Rest, the concentration of calcium at rest. Dismiss the window once you have entered the Rate Equation and Expression values. The above equation was derived in the following manner:

Flux_Pump - Flux_Leak
Flux_Pump = Vmax*Ca_C/(Ca_C+kP)
Flux_Leak = L
L is found from the balance condition at rest:
L = Flux_Pump_rest
L = Vmax*Ca_Rest/(Ca_rest + kP)
The equations can be combined to yield:
Vmax(Ca_C/(Ca_C+kP)-Ca_Rest/(Ca_Rest+kP))

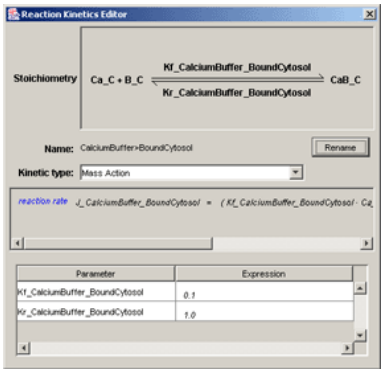
The equation is then rewritten as above with the condition that it is calculated only when Ca_C is greater than Ca_Rest.

Cytosol

Select the cytosol with the left mouse button and then use the right mouse button to select Reactions from the menu. Arrange the species so that B, Ca and CaBP are on the left side of the work area and Ca and CaBPB are on the right side of the work area. Click once on the



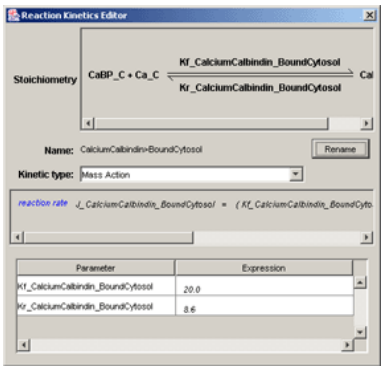
Reaction Tool,  , and then click once in the middle of the cytosol field. Use the Line Tool,  , to connect B to the left side of the Reaction Icon. Select the Line Tool again to connect Ca to the left side of



the icon. Select the Line Tool and connect CaB to the right side of the Reaction Icon.

Select the Reaction Icon and use the right mouse button to access the Properties option and open the Reaction Kinetics Editor. Press Rename to name the reaction CalciumBuffer>BoundCytosol. Select Mass Action for the Kinetic Type. Type in 0.1 for the Forward Rate and type in 1.0 for the Reverse Rate. Close the window.

Select the Reaction Icon once again and then click in the center of the Cytosol field. Use the Line Tool, selecting it each time, to connect Ca and CaBP to



the left side of the icon and CaBPB to the right side of the icon.

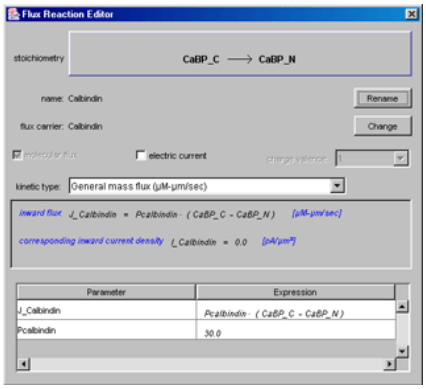
Select the Reaction Icon and use the right mouse button to access the Properties option. Press Rename to name the reaction CalciumCalbindin>BoundCytosol. Select Mass Action for the Kinetic Type. Enter 20.0 for the Forward Rate and enter 8.6 for the Reverse Rate. Close the window.

NuclearMembrane

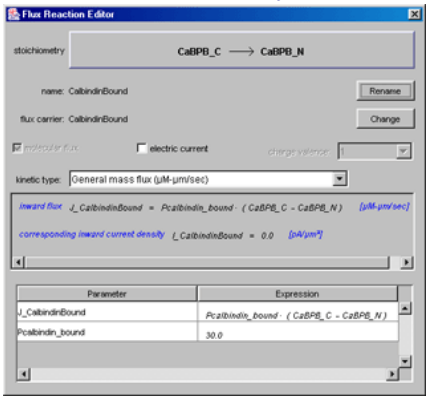
Select the NuclearMembrane with the left mouse button and then use the right mouse button to select

Reactions. Once again arrange the species such that each is visible. Set up three flux reactions; calcium, calbindin bound and calbindin. Select the Flux Icon and click once in the NM column. The Flux Reaction Editor will open automatically. Press Rename to name the flux Calcium. Press Change to select calcium for the Flux Carrier. The software will automatically connect Ca_C and Ca_N to the Flux Icon.

Select General Mass Flux for the kinetic type and define the flux, J_Calcium, with the following equation: (Pcalcium*(Ca_C-Ca_N)). Double click the expression text field for Pcalcium, the permeability coefficient for calcium, enter an expression value of 150.0. Dismiss the window once you have entered the Rate Equation and Parameter Expression.



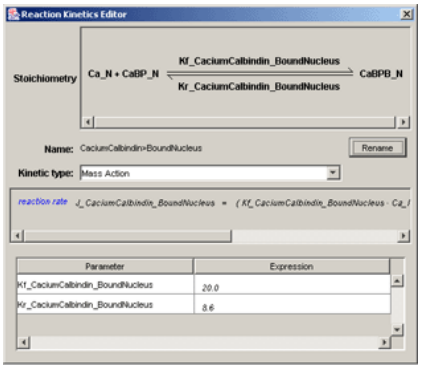
Next, add a Flux Icon in the NuclearMembrane column for Calbindin. Select the reaction icon and use the right mouse button to go to Properties>Flux Reaction Editor. Rename the flux reaction, Calbindin, and then select Calbindin as the Flux Carrier. Select General mass flux for the kinetic type and enter the following flux equation to define J_Calbindin: (Pcalbindin*(CaBP_C-CaBP_N)). Double click the Expression field for Pcalbindin, the permeability coefficient for calbindin; enter 30.0. Dismiss the window once you have entered the rate equation.



Finally, set up the flux reaction for CalbindinBound. Click once on the Flux Tool then once in the NM column. When the Flux Reaction Editor opens, rename the Flux Reaction, CalbindinBound, and select CalbindinBound as the Flux Carrier. Select General mass flux and enter the following flux Equation to define J_CalbindinBound: (Pcalbindin_bound*(CaBPB_C-CaBPB_N)). Double click the Expression text field for Pcalbindin_bound, the permeability coefficient for calbindin bound, enter 30.0 for an expression value; dismiss the window.

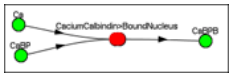
Nucleus

Select the nuclear compartment with the left mouse button and then use the right mouse button to select the Reactions option. Arrange the species such that CaBP and Ca are on the left side of the window while CaBPB is on the right side of the window. Click once on the Reaction Tool and then click once in the Reactions for Nucleus dialog placing the icon in between the species. Use the Line Tool to connect CaBP and Ca to the left of the icon and CaBPB to the right of the icon.



Select the Reaction Icon and use the Properties option to access the Reaction Kinetics Editor. Press Rename and name the reaction CalciumCalbindin>BoundNucleus. Select Mass Action for the Kinetic Type. Enter 20.0 for the Forward Rate and 8.6 for the Reverse Rate. Dismiss the window once you have entered the rates.

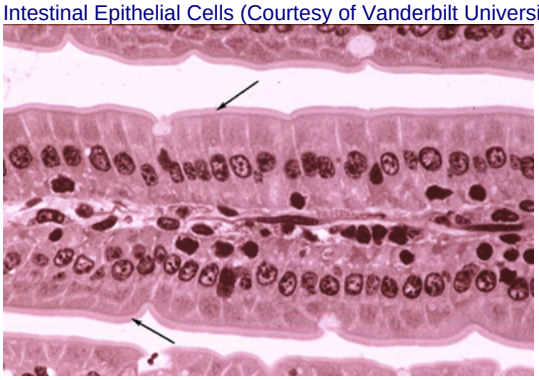
The BioModel is now complete. Resave your model and proceed to the Geometry section.



Geometry

Generation of Image for Experimental Geometry

Intestinal Epithelial Cells (Courtesy of Vanderbilt University Medical Center) and Experimental Segmented Image

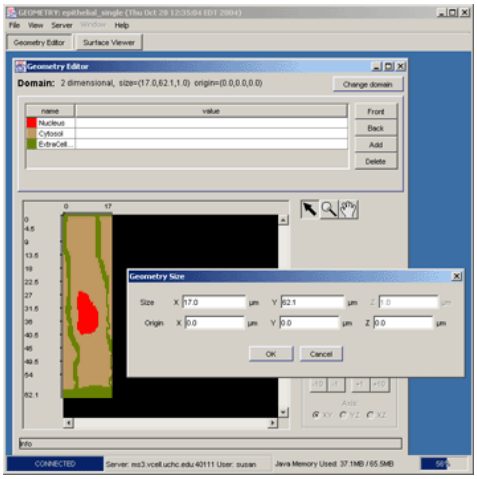


A portion of the image was used as the basis for producing the experimental geometry in this spatial simulation model. The image was slightly modified to contain clearly defined extracellular regions between neighboring cells. It was converted into a grayscale image and segmented into three distinct regions, or compartments, represented by three different pixel intensities, extracellular, cytosol and nucleus. The image was saved as a 'gif' file and stored locally until it was introduced into the public Geometry'single_epithlial.

Loading the Geometry Document

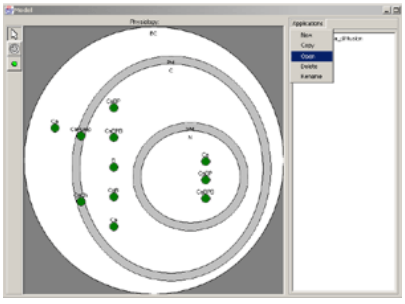
In the BioModel document, go to File>Open>Geometry>Geometry Neighborhood>tutorial>epithelial_single. The Geometry document will load with the experimental image displayed.

The image is displayed with three distinct compartments. The image size should be 17.0, 62.1, and 1.0 in the 'x,y, and z' image planes. If the image size is incorrect, click on Change domain and enter 17.0 for 'x' and 62.1 for 'y'. 'Z' should default to 1.0 since it is a single image slice.



Application

The Application is found on the Application panel of the BioModel document.



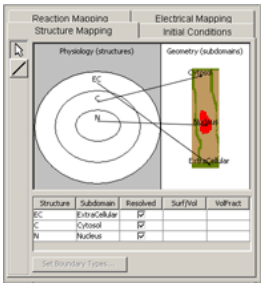
Loading the Stored Application

Select the Spatial Application once and go to Application>Open, or simply double click the Spatial Application. The Application dialog will open with the appropriate Geometry loaded and mapped according to the described BioModel. Continue with Structure Mapping below.

Creating a New Application

In the Application panel, press Application>New. Supply a name in the dialog; press OK. By default a Compartmental model is mapped to the BioModel. Press View/Change Geometry>Change Geometry. In the Geometry Neighborhood go to the tutorial folder and select epithelial_single.

Structure Mapping

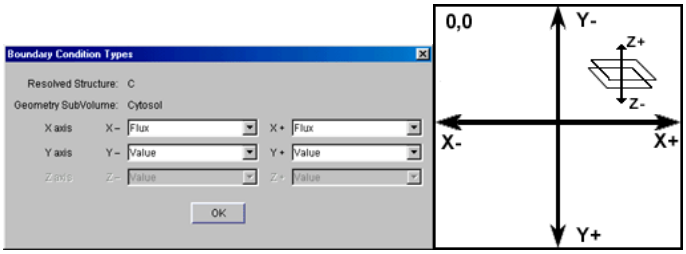


Once you have loaded the Geometry, a cartoon representing the Geometry as described in the BioModel and the Geometry image are displayed in Structure Mapping. You must manually map the Physiology structures to the Geometric subdomains using the Line Tool. You must reselect the Line Tool each time you map a new Structure to its Subdomain.

First map ExtraCellular, then Cytosol and finally Nucleus to its corresponding Subdomain. In this model each structure is spatially resolved. Membranes do not have to be physically mapped. This is performed automatically in the software as neighboring compartments are mapped. The surface to volume ratio and the volume fraction are not specified since they are resolved in the experimental geometry. You have to supply this information only if the structure is distributed.

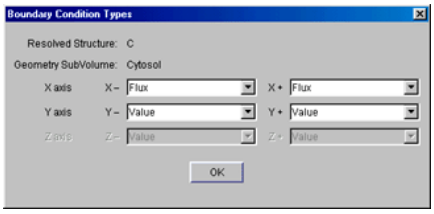
Boundary Conditions

Select ExtraCellular and press Set Boundary Types.



Use the down arrow to change the X - and X+ to Flux Boundary Conditions that represent Neumann conditions. Press OK to accept the changes and to close the dialog.

Select Cytosol and press Set Boundary Conditions. Change X- and X+ to Flux.



Leave the default Value (Dirichlet) Boundary Conditions for the nucleus.

Initial Conditions

In this section you will specify Default Concentrations and Diffusion Rates.

ExtraCellular

Double click the Initial Conditions column for Ca_EC and enter the following equation in the Expression Editor: **((1000.0*(y<0.89))+(1000.0*(y>=.89)))**, press OK. This equation explicitly defines the initial extracellular calcium concentrations in the experimental geometry.

Enter the Diffusion Rate for Ca_EC as **(300.0*((y<2.23)+(y>4.0)))** in the Expression text field for Diffusion constant; press Enter to accept your entry.

PlasmaMembrane

Double click the Initial Conditions column for CaChannel_PM. Enter the following equation in the Expression Editor: **((y<2.23)*(x>1.34)*(x<11.61))** for defining the channel regions within the

geometry and press OK. Double click the Initial Conditions column for CaPump_PM; enter the equation **(y>=57.59)** and press OK. This defines the pump regions within the geometry.

Cytosol

Enter the following values for the cytosolic species as you did for the previous species.

Species	Concentration	Diffusion Rate
Calcium	0.1	300
Calbindin	202.83	60
Calbindin Bound	47.17	60
Buffer	396.04	0.0
Buffer Bound	3.96	0.0

Nucleus

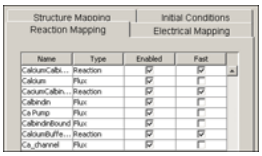
Enter the following values for the nuclear species.

Species	Concentration	Diffusion Rate
Calcium	0.1	300
Calbindin	202.83	60
Calbindin Bound	47.17	60

You do not have to enter anything in the Boundary Condition text fields. When the text fields are left blank it will default to the information entered in the Initial Conditions.

(Note: You may initially run this model with the Ca_EC concentration at 1mM as described above. This will represent a steady state model. Once that simulation is complete, you may change the concentration to 10 mM and continue to run the simulation until a new steady state is achieved. The equation for such a condition would be entered as: **((10000.0*(y<0.89))+(1000.0*(y>=.89)))**.)

Reaction Mapping



Select Fast Kinetics for the three reactions: CalciumCalbindin>BoundCytosol, CalciumCalbindin>BoundNucleus and CalciumBuffer>BoundCytosol.

Resave your BioModel at this point and proceed to Simulation.

Simulation

You can load the simulation results, from the Simulation tab, or create your own simulation. If you choose to use the simulation and results that are there, it is still recommended to follow the remainder of the tutorial so you will familiarize yourself with the software.

Using the Saved Simulation

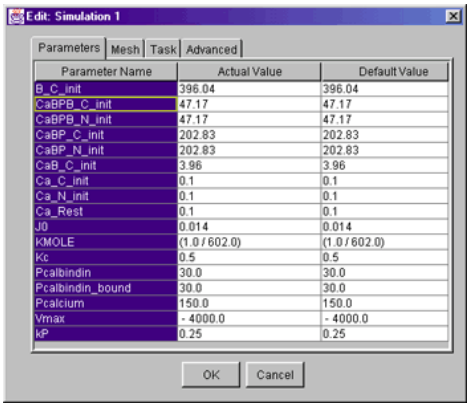
Select the simulation in the Simulation panel and press Edit. See Parameters below for more information.

Creating a New Simulation

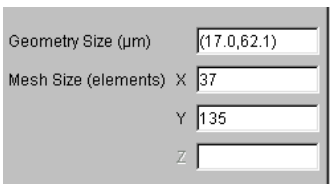
Press New in the Simulation dialog, a default simulation will be created. Double click the Simulation Name text field to enter the name. You can enter descriptive notes about the simulation in the Comments section, under the Simulation Summary. Press Edit to access the additional run conditions for the simulation.

Parameters

Select the Parameters tab. You can change the initial values of the parameters by double clicking in the Actual Value column for the particular Parameter. Type in a value and either press Enter or change your selection. Note, values that have changed from the original value show up in red text. For the initial simulation you will not make any changes here.



Mesh



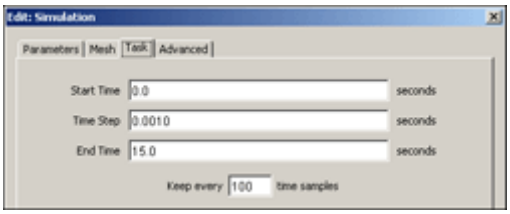
Select the Mesh tab. The Mesh tab lists the Geometry size in microns and the Mesh size in elements. By default, the Mesh Size is equal to 37 X 135 pixels. Double click in the X and Y text fields and change the

mesh size to 39 x 140.

The mesh size is larger than the actual image size. This is done to ensure that a full mesh element is captured during the calculations instead of half of a mesh element. The Geometry size is obtained from the segmented image that was entered into the Virtual Cell image database. This size cannot be edited here.

Task

Select the Task tab. Leave the Start Time at 0, change the Time Step to .001 and End Time to 15. Change the save interval to Keep every 100 time samples.



You can press OK at the bottom of the dialog at this point. You do not have to worry about the Advanced tab for this example. The Advanced tab offers additional options for ODE solvers, as well as some additional features for PDE simulations, but for this example you will not use them.

You are ready to run a simulation at this point. Make sure the simulation is still selected, and then press Run to start the simulation. The software will automatically resave the BioModel and initiate the simulation. As the simulation runs, the Results button will become enabled. Press the button to see the results that have been generated thus far. Alternatively you can wait until the simulation is complete and the Results dialog will open automatically.

See Chapter 8 of the User Guide for more information about viewing simulation results.